The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Information Technology

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

SPECTINOMYCIN
(cattle, pigs and poultry)

SUMMARY REPORT (3)

1. Spectinomycin is an aminocyclitol antibiotic produced by *Streptomyces spectabilis*. It exerts its bacteriostatic effect by binding to the 30S subunit of bacterial ribosomes and inhibiting the translation of protein synthesis. Spectinomycin is indicated for use via the oral and parenteral routes, often in conjunction with lincomycin, in the treatment of a variety of enteric, respiratory and other infections of cattle, sheep, pigs and poultry. In veterinary medicine spectinomycin is used as hydrocloride and sulphate salts. In cattle including lactating cows, products are given by intramuscular injection at a dosage of 30 mg/kg bw/day or subcutaneous injection at 15 mg/kg bw/day, in both cases for 5 consecutive days. In pigs, spectinomycin is administered in the diet at 22 mg/kg feed for 21 consecutive days or as an intramuscular injection of 11 mg/kg bw. In chickens, spectinomycin is administered orally in drinking water and feed at doses equivalent to 100 mg/kg bw/day for 3 to 7 days.

   Spectinomycin is currently entered into Annex III of Council Regulation (EEC) No 2377/90 in accordance with the following tables:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Spectinomycin	Spectinomycin	Bovine, porcine, poultry	300 µg/kg 500 µg/kg 2000 µg/kg 5000 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 01.07.2000
		Bovine	200 µg/kg	Milk	

Pharmacologically active substance(s)	Marker residue	Animal Species	MRLs	Target tissues	Other provisions
Spectinomycin	Spectinomycin	Ovine	300 µg/kg 500 µg/kg 2000 µg/kg 5000 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 01.01.2002. Not for use in animals producing milk for human consumption.
		Chicken	200 µg/kg	Eggs	

   Further data were provided to support the establishment of final MRLs for spectinomycin in respect of cattle, pigs and poultry.

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2. Pharmacokinetic data indicate that absorption is poor via the oral route in humans and in animals, but rapid and extensive after intramuscular injection. After oral administration of 100 and 500 mg/kg bw to dogs, mean peak serum concentrations were around 22 µg/ml and 80 µg/ml, respectively. Spectinomycin is not extensively bound to proteins either in serum or in milk. Plasma elimination half lives range from 1 to 3 hours in various species (sheep, cattle, dogs and humans) when administered via the oral, intramuscular and intravenous routes. Following an intramuscular dose of 2 g or 4 g, the C_{max} values in humans were approximately 103 mg/l, 1 hour after treatment and 160 mg/l, 2 hours after treatment, respectively. The half-life of plasma elimination ranged from 1.2 to 2.8 hours. After parenteral administration, spectinomycin is rapidly and extensively excreted in the urine. In humans, 70 to 100% of the administered dose is excreted unchanged in the urine within 48 hours after treatment. After oral administration, excretion is primarily in the faeces. The data available suggest that spectinomycin is not extensively metabolised in animals or in humans. In some studies in cattle, the metabolite dihydrospectinomycin, which has 10% of the microbiological activity of spectinomycin, was found in liver.

3. Spectinomycin has low acute toxicity in mice, rats and dogs when administered by various routes. The acute oral and subcutaneous LD_{50} values were greater than 5000 mg/kg bw in rats. No signs of toxicity were observed in dogs given single doses of up to 270 mg/kg bw.

4. In numerous repeated dose studies where spectinomycin was administered by injection (intramuscular, intravenous, subcutaneous) the only significant findings were injection site reactions. Spectinomycin is irritant on intramuscular injection. Groups of TUC/SPD rats were given daily oral gavage doses of a 1:1 mixture of spectinomycin:lincomycin for 90 days. The most notable finding was a change in the consistency of the faeces. There were some minor changes in clinical chemistry parameters in the groups administered 300 and 1000 mg/kg bw/day but these did not correlate with any pathological changes. The NOEL was 100 mg/kg bw/day spectinomycin:lincomycin, equivalent to 50 mg spectinomycin/kg bw/day. Groups of Beagle dogs were given daily oral doses of 0, 100, 250, 500, 750 or 1000 mg spectinomycin/kg bw/day, in gelatin capsules, for 28 days. The only observed effect was an increase in soft faeces at the top dose level. The NOEL was 750 mg/kg bw/day. In another study, groups of Beagle dogs were given daily oral doses of 0, 100, 300 or 1000 mg/kg bw/day of a 1:1 mixture of spectinomycin:lincomycin, in gelatin capsules, for 90 days. Intermittent diarrhoea and soft faeces were the only significant findings. The NOEL was 100 mg/kg bw/day spectinomycin:lincomycin, equivalent to 50 mg spectinomycin/kg bw/day.

5. In a 3-generation reproduction study in Sprague-Dawley rats, doses equivalent to 0, 100, 200 or 400 mg/kg bw/day were administered in the diet. There were no adverse effects on reproductive parameters up to the highest dose administered. Hepatocellular changes, notably clumped basophilic material in the cytoplasm and hepatocellular swelling was noted in some animals in the F_{1b} generation. The NOEL was 100 mg/kg bw/day.

6. Developmental toxicity was examined in mice, rats and rabbits. There was no evidence of teratogenicity or foetotoxicity in two studies in which pregnant ICR mice were treated intraperitoneally from days 7 to 12 of gestation, using dose levels of 0, 400 or 1600 mg/kg bw/day. There was no evidence of teratogenicity or foetotoxicity after oral administration of 0, 100 or 300 mg/kg bw/day to pregnant TUC/SPD rats or following oral administration of 0, 100, 300, 1000 or 3000 mg/kg bw/day to pregnant Sprague-Dawley rats from days 6 to 15 of gestation. The substance was not teratogenic in further studies in rats using intraperitoneal administration (0, 400 or 1600 mg/kg bw/day) or subcutaneous administration (0, 100, 300 mg/kg bw/day). In rabbits, litter size and weight were reduced following subcutaneous administration of 150 and 300 mg/kg bw/day from days 8 to 18 of gestation, but there was no evidence of teratogenicity. The substance was not teratogenic in two further studies in rabbits using intramuscular administration (0, 100 and 300 mg/kg bw/day and 0 and 100 mg/kg bw/day).

7. Spectinomycin was not mutagenic in a range of well conducted *in vivo* and *in vitro* tests. These included *in vitro* assays for gene mutation in *Salmonella typhimurium* TA98a, TA98, TA100, TA102, TA1535, TA1537 and TA1538 and in Chinese Hamster Ovary cell lines CHO-K1-BH4 (HPRT locus) and AS52 (XGPT locus). Negative results were also obtained in an *in vitro* unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes and in an *in vitro* chromosomal aberration assay in human lymphocytes. In an *in vivo* bone marrow micronucleus test *g*roups of Sprague-Dawley rats were given intraperitoneal doses of 750, 1500 or 3000 mg spectinomycin/kg bw, as 2 half doses, 24 hours apart and killed 30 or 48 hours after the first dose. CD-1 mice were given intraperitoneal doses of up to 2500 mg/kg bw and killed 24, 48 or 72 hours later. The incidence of micronucleated polychromatic erythrocytes was not increased in either study.

8. No carcinogenicity studies were carried out. Such studies were not considered necessary due to the negative results in the mutagenicity assays, the absence of structurally-alerting features and the lack of pre-neoplastic lesions in the repeated dose toxicity studies.

9. Groups of cats were given daily intramuscular injections of 0, 30, 60 or 120 mg/kg bw/day for 75 to 90 days. Tests for cochlear function were carried out twice weekly. No abnormalities were found and there was no evidence of reduced eighth nerve function. In another study, tests for cochlear and vestibular function indicated no signs of ototoxicity in 15 healthy male volunteers given daily intramuscular injections of 8 mg spectinomycin/day, (equivalent to 130 mg/kg bw/day) for 21 days.

10. Spectinomycin is used in human medicine for the treatment of gonorrhoea. It is normally administered by intramuscular injection to adults at a dose of 2 g or 4 g or to children at a dose of 40 mg/kg bw. The most common adverse reactions were reported to be soreness at the injection site, urticaria, dizziness, nausea, chills and fever. Anaphylaxis was reported to be rare. Spectinomycin did not cross react with penicillins in human clinical trials.

11. The repeated dose toxicity studies conducted on spectinomycin were performed using the substance alone or in combination with lincomycin. The lowest NOEL identified using spectinomycin alone was 100 mg/kg bw in a rat dietary reproduction study. In combination with lincomycin, the lowest NOEL was 50 mg spectinomycin/kg bw in 90-day studies in rats and dogs. A conservative toxicological ADI of 0.25 mg/kg bw can be established, using a safety factor of 200 to account for the combined use of the substances.

12. The potential for adverse effects on the human gut flora was studied *in vitro* in a wide range of organisms including both animal and human pathogens. The MIC data for a number of bacterial species representative of the anaerobic flora in humans was examined including *Bacteroides*, *Peptostreptoccus*, *Fusobacterium*, *Eubacterium*, and *Clostridium* spp. Many had a MIC_{50} of greater than 50 µg/ml. *Bifidobacterium* were more sensitive with MIC values for spectinomycin in the range of 2 to 32 µg/ml. The modal MIC was 16 µg/ml with an inoculum density of 10^6 and 8 µg/ml with an inoculum density of 10^4. The value of 16 µg/ml was used to calculate the microbiological ADI.

14. For the assessment of the microbiological risk, use was made of the formula recommended by the CVMP:

$$\text{ADI (µg/kg bw)} = \frac{\dfrac{MIC_{50}\text{ most sensitive organism} \times CF2}{CF1}\ (\text{µg/ml}) \times \text{daily faecal bolus (150 ml)}}{\text{fraction of an oral dose available for microorganisms} \times \text{weight of human (60 kg)}}$$

Based on the above formula, the microbiological ADI can be calculated as follows:

$$ADI = \frac{\frac{16 \times 1}{1} \times 150}{1 \times 60} = 40\ \mu g/kg\ bw\ i.e. = 2400\ \mu g/person$$

The following assumptions were made:

- CF1 = 1 because the modal MIC_{50} of the most sensitive organism (*Bifidobacterium*) was used, and there was no evidence of plasmidic resistance,
- CF2 = 1 because no data on differences of *in vitro* and *in vivo* growth conditions were provided to justify a higher value,
- 150 g was the weight of the daily faecal bolus,
- a bioavailability factor of 1 because absorption is very poor after oral administration.

15. A study was conducted to investigate the effects of spectinomycin on acid production on dairy starter organisms using 13 relevant strains of *Lactococcus*, *Leuconostoc*, *Streptococcus*, *Lactobacillus* and *Bifidobacterium*. The overall no-observable effect concentration based on the most sensitive strain tested (*Lactobacillus acidophilus*) was 400 ng/ml. This value corresponds to 388 µg/kg, corrected for density (1.032).

16. Spectinomycin was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1994. The Committee did not set a toxicological ADI but established a microbiological ADI of 40 µg/kg bw.

17. Pharmacokinetic studies were conducted in cattle and pigs.

After administration of 10 mg spectimomycin/kg bw to cattle by intramuscular, intravenous, and subcutaneous routes the areas under the curves (AUC) were 65, 77 and 77 µg·h/ml, respectively. After intramuscular and subcutaneous administration C_{max} values of 27 and 20 µg/ml were obtained at t_{max} times of 0.6 and 1.1 hours after treatment.

In another study in cattle, after subcutaneous administration of 15 mg/kg bw/day of ^{3}H-spectinomycin for 5 days, elimination in urine and faeces amounted to 69% and 8% of the administered dose 1 day after treatment and 77% and 8% of the administered dose 15 days after treatment. The total residue content of tissues were determined in cattle killed 1, 5, 10 and 15 days after the last dose. One day after treatment the highest residue concentrations were found in kidney (59 600 µg equivalents/kg) followed by liver (32 400 µg equivalents/kg), fat (1270 µg equivalents/kg) and muscle (1030 µg equivalents/kg). The total residue concentrations in kidney, liver, fat and muscle were: 14 200, 18 800, 1060 and 360 µg equivalents/kg at 5 days; 4500, 7540, 830 and 360 µg equivalents/kg at 10 days and 2660, 4540, 770 and 290 µg/kg at 15 days. Eight metabolites were identified in urine of which spectinomycin accounted for 63% of the total radioactivity present. On days 1, 5, 10 and 15 after treatment spectinomycin accounted for 4.2, 3.1, 2.5 and 3.4% of the total residue in liver and 15.3, 11.9, 9.4 and 6.6% of the total residue in kidney. On days 1, and 5 after treatment spectinomycin accounted for 20.6 and 22.5 % of the residue with antimicrobial activity in liver and all of the residue with antimicrobial activity in kidney. The residues in muscle and fat were too low for characterisation and were below the limit of quantification of the microbiological assay (about 100 µg/kg).

In pigs, a single intramuscular injection of 10 mg spectinomycin (plus 5 mg lincomycin)/kg bw resulted in a plasma C_{max} of 28 µg/ml at a t_{max} of 0.4 hours and a half-life of elimination of 1 hour. Plasma concentrations were less than 1 µg/ml after 6 hours and less than 0.032 µg/ml by 16 hours after treatment. When pigs were fed spectinomycin in the feed at a concentration of 44 mg/kg feed for 8 days, the spectinomycin concentrations in plasma were below the limit of quantification (0.1 µg/ml) of the HPLC method, indicating poor absorption via the oral route.

In another study in pigs after intramuscular administration of 10 mg/kg bw spectinomycin as the hydrochloride or sulphate salt, the area under the curves (AUC) were 88.7 and 107.6 µg·h/ml respectively. The C_{max} values were 43.1 and 47.7 µg/ml obtained at t_{max} times of 0.4 and 0.45 hours, respectively. Two pigs were killed at day 1, 2 and 5 after treatment and the residues of spectinomycin in kidney and injection site muscle were determined by HPLC. The limit of quantification was 100 µg/kg. When the sulphate as opposed to the hydrochloride salt was administered, the spectinomycin concentrations were consistently higher (by approximately 20%) in kidney but no difference was apparent in the magnitude of the residues at the injection sites.

18. When cattle were intravenously injected with 20 mg spectinomycin/kg bw, the peak plasma concentrations ranged from 52 to 71 µg/ml with an average elimination half-life of 1.2 hours. The pharmacokinetics of spectinomycin in cattle were similar after intramuscular and intravenous injection (equal areas under the curve) and appeared unaltered by the presence of lincomycin. After administration of an intramuscular dose of 22 mg ^{3}H-spectinomycin/kg bw in cattle 56% of the dose was eliminated in the urine within 24 hours after treatment.

19. Twelve hours after a pig was orally dosed with 44 mg ^{3}H-6'methyl-spectinomycin, 79% of the radioactivity remained in the gastrointestinal tract, 0.05% was recovered from faeces and 4.5% from urine. Within 24 hours of an intramuscular injection of 10 mg/kg bw, over 55% of the administered radioactivity was recovered from the urine as unchanged spectinomycin. In another study in which pigs were administered 10 mg/kg bw spectinomycin, there was no statistical difference in the plasma concentrations determined using HPLC and using a microbiological assay, indicating that spectinomycin was the major microbiologically active component in plasma, for the first 8 hours after treatment.

20. Non-ruminating calves were given intramuscular doses of 30 mg spectinomycin/kg bw/day for 5 consecutive days and groups of 4 animals were killed on days 1, 3, 7, 10 and 14 after the last (5th) dose. Tissue samples were analysed by a validated analytical method based on HPLC with ultra violet (UV) detection. The average spectinomycin concentrations in liver, kidney muscle and fat were 6 410, 106 000, 1150 and less than 250 µg/kg 1 day after the last dose. These residue concentrations depleted as follows: 4654, 43 053, 646, and less than 200 µg/kg at 3 days, 1549, 9547, 357 and less than 28 µg/kg at 7 days, 1373, 4179, 251 and less than 28 µg/kg at 10 days and 903, 2750, 200 and less than 27 µg/kg at 14 days after the last dose. Over the same time period injection site muscle spectinomycin concentrations depleted from 19 200 to 1310 µg/kg.

21. Cattle were subcutaneously treated with 15 mg spectinomycin/kg bw/day for 5 consecutive days and groups of 4 cattle were slaughtered at 5, 10, 15 and 20 day time points after the last dose. The spectinomycin concentration in tissues were determined using high performance liquid chromatography (HPLC) with a limit of quantification (100 µg/kg) for bovine tissues. The spectinomycin concentrations in all injection site samples were below the limit of quantification at all time points. Spectinomycin concentrations were highest in kidney: mean concentrations after treatment were 3970, 950, 270 and 160 µg/kg on days 5, 10, 15 and 20 respectively. The average spectinomycin concentrations in liver, muscle and fat were 280, 230 and 380 µg/kg at 5 days after treatment, 80, 150, and 140 µg/kg at 10 days after treatment, less than 100, 130, and 200 µg/kg at 15 days after treatment and less than 100, 130 and 200 µg/kg at 20 days after treatment.

22. In another study, cattle were subcutaneously injected with 15 mg spectinomycin/kg bw/day for 5 days and groups of 4 animals killed on days 1, 2, 3, 5 and 10 after treatment. The residues of spectinomycin in tissues were determined by HPLC and by a microbiological assay. The highest spectinomycin concentrations were found in kidney (17 900, 9420, 6750, 4340, and 1090 µg/kg on days 1, 2, 3, 5 and 10 after treatment, respectively) and accounted for all of the residue with antimicrobial activity. The spectinomycin concentrations in liver (1180, 670, 540, 550 and 160 µg/kg on days 1, 2, 3, 5 and 10 after treatment, respectively) accounted 32% of the total residue with antimicrobial activity. The spectinomycin concentrations in muscle (remote from the injection site) were 420, 380, 340, 260 and 120 µg/kg and at the injection site were 1970, 1230, 1050, 780 and 360 µg/kg on days 1, 2, 3, 5 and 10 after treatment, respectively. For muscle, the ratio of the residues of spectinomycin to the residue with antimicrobial activity could not be assessed as the concentrations were below the limit of quantification of the microbiological assay (2000 µg/kg).

23. In a radiometric residue depletion study, pigs were fed 44 mg ^{3}H-spectinomycin (plus 44 mg lincomycin)/kg feed, equivalent to approximately 2.8 mg spectinomycin/kg bw, for 7 days and groups for 4 animals killed on days 0, 1, 3, 7 and 10 after the end of treatment. Total residues in tissues were determined by liquid scintillation counting (LSC) before and after lyophilisation (i.e. for tritiated water correction). Immediately after treatment the highest residue concentrations were found in kidney followed by liver, fat and muscle (635, 238, 155 and 5 µg equivalents/kg respectively). The total residue concentrations in kidney, liver, fat and muscle were 460, 138, 135, and less than 5 µg equivalents/kg 1 day after treatment; 240, 100, 168 and less than 5 µg equivalents/kg 3 at days after treatment; 65, 60, 170 and less than 5 µg equivalents/kg at 7 days and less than 5 µg equivalents/kg in all tissues with the exception of fat (145 µg equivalents/kg) at 10 days after treatment.

    In another study, piglets were fed twice daily with a diet containing spectinomycin equivalent to a dosage of about 20 mg spectinomycin/kg bw/day for 5 days then groups of 4 animals were killed on days 1, 3, 7, 10 and 14 day after the last (10 th) dose. The spectinomycin concentration in tissues were determined by a validated analytical method based on HPLC. The average spectinomycin concentrations in liver, kidney muscle and skin + fat were 2150, 18 100, 604 and 694 µg/kg at 1 day. These residue concentrations depleted as follows: 1030, 7700, less than 300 and less than 394 µg/kg at 3 days, 399, 4411, less than 47 and less than 250 µg/kg at 7 days, less than 198, 1899, less than 47 and less than 250 at 10 days and less than 198, less than 500, less than 300 and less than 26 µg/kg at 14 days after treatment.

24. Further studies were carried out in which pigs were dosed using intramuscular administration. In one study, pigs were given 3 intramuscular injections per day of a combination product at a dose of 10 mg spectinomycin/kg bw/day and 5 mg lincomycin/kg bw/day. The pigs were slaughtered (3 per time-point) and residues in tissues were determined using a microbiological assay, using and *Escherichia coli* strain particularly sensitive to spectinomycin, with a limit of detection of 1000 µg/kg. One day after the last dose, mean residues in kidney and liver were 28 370 and 1360 µg/kg and residues in muscle and fat were below the limit of detection. The mean residues in kidney depleted to 8110 µg/kg, 3 days after the last dose, and to 5090 µg/kg, 5 days after the last dose. Three days after the end of treatment, residues in one sample of liver were below the limit of detection and were 1100 µg/kg and 1960 µg/kg in the two other samples. In another study, HPLC thermospray mass spectrometric procedure was used to determine residues of spectinomycin in kidney samples. The kidney samples were taken from groups of 2 pigs which were slaughtered 12, 24, 48 and 96 hours after intramuscular administration at 10 mg/kg bw. The mean residues were 13000, 6950, 3400 and 1300 µg/kg respectively. Comparison of the results from the 2 studies indicated that spectinomycin accounted for approximately 25 and 20% of the residues in kidney and liver with antimicrobiological activity.

25. Broiler chickens were *ad libitum* fed drinking water containing spectinomycin at a dose equivalent to 50 mg spectinomycin/kg bw/day for 5 consecutive days then killed in groups of 6 on days 1, 4, 7, 11 and 14 after treatment. Tissue samples were analysed by a validated method based on HPLC with UV detection. The spectinomycin concentration in tissue samples from birds at all time points were below their the limits of quantification of the analytical method (500 µg/kg for liver and kidney, 250 µg/kg for muscle and skin + fat).

26. In another study, chickens were treated via drinking water with approximately 100 mg spectinomycin (and 50 mg lincomycin)/kg bw for 7 days and groups for 12 birds (6 pairs) killed on days 0, 0.25, 0.5, 1, 2, 4 and 8 after treatment. The concentrations of spectinomycin in tissues from paired birds were determined by HPLC. Immediately after treatment the highest spectinomycin concentrations were found in skin + fat, followed by kidney, liver and muscle (2850, 1950, 433, and 483 µg/kg, respectively). The spectinomycin concentrations in skin + fat, kidney, liver, and muscle were: 1680, 1390, 383, and 250 µg/kg at 6 hours; 1317, 967, 267 and 267 µg/kg at 12 hours; 683, 550, 217 and 133 µg/kg at 1 day; 571, 683, 150, and less than 100 µg/kg at 2 days; 240, less than 100, less than 100 and 133 µg/kg at 4 days and 425, less than 100, less than 100 and less than 100 µg/kg at 8 days.

27. Broiler chickens were treated via drinking water with approximately 704 mg spectinomycin/kg bw (i.e. 1000 mg/kg lincomycin:spectinomycin 1:2 w/w) for 7 consecutive days. Groups of 18 birds were killed on treatment days 3, 5 and 7 and after treatment on withdrawal days 1, 3, 5, 7, 10 and 14. The residues in tissues were measured by a microbiological assay sensitive to spectinomycin. After the end of treatment, the residues in skin + fat, kidney, liver, and muscle were: less than 1200, 44 300, 5500 and less than 1000 µg/kg at 1 day; less than 1100, 14 900, 2800, and less than 1 000 µg/kg at 3 days; less than 1100, 3500, less than 2200, and less than 1000 µg/kg at 5 days; less than 1000, 2100, less than 1100 and less than 1000 µg/kg at 7 days; and less than 1000 µg/kg in all relevant tissues at later time points.

28. Because of the different dosage regimens used in the studies in chickens, it was difficult to draw firm conclusions about the relationship between residues of spectinomycin and total residues with microbiological activity. Extrapolation suggested that spectinomycin accounted for 28 to 31% of residues with microbiological activity in chicken liver and 10 to 30% of the residues in chicken kidney, for up to 24 hours after treatment.

29. Lactating cows (2 groups: mid-lactation producing 30 to 35 litres of milk per day and late-lactation producing 17 to 20 litres per day), were given intramuscular doses of 30 mg spectinomycin/kg bw/day for 5 consecutive days and milk samples were collected twice daily (at 12-hour interval) for 10 consecutive days after the last (5th) dose. Milk samples were analysed by a validated method based on HPLC with UV detection. In cows in mid-lactation, the average spectinomycin concentrations were 1431 (range 88 to 2077), 439 (range 213 to 899) and less than 100 (range less than 100 to 130) µg/kg in 12, 24 and 36 hour milk samples, respectively. In late lactation cows the average spectinomycin concentrations were 1748 (range 1133 to 2109), 469 (range 308 to 621), 121 (range less than 100 to 156) µg/kg in 12, 24 and 36 hour milk samples respectively. All milk samples collected from mid- and late-lactation cows at time points at or after 48 hours contained spectinomycin concentrations below 100 µg/kg.

30. In another study, 12 cows were injected intramuscularly and then intravenously with 20 mg spectinomycin/kg bw/day for 4 days. Residues in milk were determined using a microbiological assay with a limit of detection of 700 µg/kg. Milk samples were assayed from 0 to 120 hours after treatment. Residues were below the limit of quantification by 24 hours after each injection. Although the data for milk did not permit an estimation of the ratio of spectinomycin residues to total residues with microbiological activity, the data confirmed that spectinomycin was an appropriate marker residue for milk and accounted for a substantial proportion of the residues in milk. Although these studies were carried out using different protocols these data were considered sufficient to conclude that spectinomycin accounts for all of the residue with antimicrobial activity in milk.

31. JECFA, at their 32nd meeting, assessed the same data as submitted to the CVMP and established the following MRLs: 2000 µg/kg for liver, 5000 µg/kg for kidney, 500 µg/kg for muscle, 2000 µg/kg for fat or skin + fat, 200 µg/kg for milk and 2000 µg/kg for eggs in all relevant target species.

32. Several analytical methods for the determination of residues of spectinomycin have been described in the ISO 78/2 format. One method involved an HPLC procedure employing a gradient elution for separation with post column oxidation and derivatisation with o-phthalaldehyde to a permit quantification by fluorescence detection. Specificity was satisfactory and residues of 7 other antibiotics did not interfere in the analysis. The limits of quantification were 100 µg/kg for bovine, porcine and chicken muscle, 100 µg/kg for bovine fat, 250 µg/kg for porcine skin + fat, 100 µg/kg for chicken skin + fat, 100 µg/kg for bovine liver, 1000 µg/kg for porcine and chicken liver, 100 µg/kg for bovine kidney, 2500 µg/kg for porcine kidney and 2000 µg/kg for chicken kidney. For some tissues, there was no information on intermediate precision at the limit of quantification. Another HPLC method involved derivatisation with dinitrophenylhydrazine followed by quantification by UV detection. Specificity was satisfactory and residues of gentamicin and lincomycin did not interfere in the assay. The limits of quantification were 500 µg/kg for bovine, porcine and chicken, liver and kidney, 250 µg/kg for bovine fat and porcine and chicken skin + fat, 150 µg/kg for bovine muscle, 300 µg/kg for porcine muscle, 250 µg/kg for chicken muscle, and 100 µg/kg for bovine milk. A confirmatory method employing HPLC and atmospheric pressure chemical ionisation (APCI) collision induced dissociation (CID) mass spectrometry was also described. The limited validation data available for this method indicated a limit of quantification of 100 µg/kg for bovine kidney.

Conclusions and recommendation

Having considered that:

- a microbiological ADI of 40 µg/kg bw (i.e. 2400 µg/person) was established,
- spectinomycin was identified as the marker residue and represents about 20% and 100% of total residue with antimicrobial activity in bovine liver and kidney, respectively for up to 5 days after the end of treatment; residues in muscle and fat were too low for the percentage in these tissues to be determined,
- the data for pigs and chickens were less robust but indicated that spectinomycin could conservatively be estimated to represent approximately 20% of the total residues with antimicrobiological activity in liver and kidney, residues in muscle and fat were too low for the percentage in these tissues to be determined,
- spectinomycin was an appropriate marker residue for milk and accounted for all of the residues with antimicrobiological activity,
- validated routine analytical methods for determining the marker residue in the edible tissues of the target species and in bovine milk are available,
- it is not possible to retain the MRLs established by JECFA because this would result in consumer intake exceeding the ADI,
- a no-observable effect concentration of 388 µg/kg was determined for the most sensitive strain of dairy starter organism (*Lactobacillus acidophilus*);

the Committee for Veterinary Medicinal Products recommends the inclusion of spectinomycin in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal Species	MRLs	Target tissues	Other provisions
Spectinomycin	Spectinomycin	Bovine	300 µg/kg 500 µg/kg 1000 µg/kg 5000 µg/kg 200 µg/kg	Muscle Fat Liver Kidney Milk	
		Porcine, chicken	300 µg/kg 500 µg/kg 1000 µg/kg 5000 µg/kg	Muscle Skin + Fat Liver Kidney	

Based on these MRLs values the daily intake when considering pig meat, milk and eggs will represent about 91% of the ADI.